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Association of a Polymorphism in Intron 13 of the Monoamine Oxidase B Gene With Parkinson Disease

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Monoamine oxidase B (MAO-B) is an enzyme that has relevance for Parkinson disease (PD) because of its roles in catabolizing dopamine and potentially activating exogenous neurotoxicants. A polymorphism of the gene encoding MAO-B has been identified as a single base change (A or G) in intron 13 of the X chromosome. The A allele was previously associated with an approximately twofold risk of PD. The present study compared A and G allele frequencies between newly diagnosed idiopathic PD cases and a control group free of neurodegenerative diseases. All study subjects were Caucasian. Cases were 37 men and 25 women, age 37–80 years; controls were 50 men and 29 women, age 45–82 years. MAO-B genotype was determined by the allele-specific polymerase chain reaction on DNA extracted from peripheral lymphocytes. In complete contrast to previous studies, elevated risks were detected with the G allele. The age-adjusted odds ratio for the G allele in males was 1.87 ((95% confidence interval) 0.78–4.47). Among females the age-adjusted odds ratios were 5.00 ((95% confidence interval) 1.13–22.1) for the GA genotype and 5.60 ((95% confidence interval) 1.01–30.9) for the GG genotype. These findings, although of limited statistical precision, suggest that the G allele of this MAO-B polymorphism may relate to PD risk. Am. J. Med. Genet. 74:154–156, 1997.

KEY WORDS: genetic polymorphisms; monoamine oxidase; Parkinson disease

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INTRODUCTION

Monoamine oxidase (EC 1.4.3.4) is a membrane-bound mitochondrial enzyme that exists in two forms, A and B. Type B monoamine oxidase (MAO-B) is potentially relevant in Parkinson disease (PD) because of its roles in catabolizing dopamine and other biogenic amines [Riederer et al., 1989], and in potentially activating exogenous neurotoxicants similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the agent found to induce Parkinsonism among intravenous users of synthetic heroin [Chiba et al., 1984]. Moreover, inhibition of MAO-B activity in the brains of cigarette smokers [Fowler et al., 1996] may be a pharmacological explanation for the inverse risk found with smoking in some studies [Grandinetti et al., 1994].

The gene encoding for MAO-B is located on the X chromosome, and is composed of 15 exons and 14 introns, spanning 60 Kb [Hsu et al., 1989]. Previous efforts to identify genetic polymorphisms of the MAO-B gene that may predict PD susceptibility have focused on intron sequences. No differences between PD cases and controls were found for GT repeating sequences of intron 2 in two studies [Konradi et al., 1992; Ho et al., 1994]. Nanko et al. [1996] recently analyzed eight allelic forms of intron 2 and reported no consistent association with PD among Japanese subjects. However, a twofold PD risk elevation was associated with a polymorphism of intron 13, detected by single-strand conformational polymorphism analysis [Kurth et al., 1993]. This polymorphism was subsequently determined to be a single base difference (A or G), in which the A allele occurred more frequently among PD cases than controls in Caucasians [Ho et al., 1995]. In contrast, the A allele was found to be twice as common overall in the Japanese population, but was unrelated to PD [Morimoto et al., 1995]. We undertook the present study to determine whether the association of PD with the A allele of the MAO-B intron 13 was evident

among Caucasian subjects from a population-based case-control study of PD.

MATERIALS AND METHODS

Study Subjects

Study subjects were identified from the membership of a large health maintenance organization, Group Health Cooperative (GHC), in western Washington state. Idiopathic PD cases newly diagnosed during 1992–1995 included 37 males and 25 females (age 37–80). All cases exhibited at least two of the four cardinal signs of PD: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria included use of certain medications (e.g., phenothiazines, haloperidol) during the 12 months preceding symptom onset, prior history of multiple cerebrovascular events, or another explanation for Parkinsonism symptoms (e.g., brain injury, brain tumor, encephalitis). Case charts were reviewed for diagnostic verification independently by two non-GHC neurologists (G.M.F. and P.D.S.). Past medical conditions were ascertained from chart review and patient interview.

Control subjects free of progressive neurologic disorders, determined from chart reviews and patient interviews, were identified from the roster of GHC members. Controls were matched to cases by age (within 5 years), gender, original year of enrollment in GHC, and clinic. We identified 50 male and 29 female controls (age 45–82) who met inclusion criteria. All subjects were non-Hispanic Caucasians. Subjects were informed of the study objectives and procedures by an introductory letter, followed by a telephone contact. Informed consent was provided with forms approved by the Human Subjects Review Committees of the University of Washington and the GHC Center for Health Studies.

Genetic Polymorphism Assay

DNA was extracted from peripheral blood leukocytes according to Grimberg et al. [1989]. The analysis of genotype G or A in intron 13 was performed by allele-specific polymerase chain reaction (PCR) using the G- and A-specific oligonucleotides (5′-CACTGGCAAATAGCAAAAGT-3′ and 5′-CACTGGCAAATAGCAAAAGC-3′) described by Ho et al. [1995] as reverse primers, together with a forward primer complementary to a 5′ region in exon 13 and corresponding to the MAOB3FP primer described by Kurth et al. [1993]: 5′GGATTTACTTTGCAGGCACC-3′. PCR products (663 bp long) obtained from 3 representative individuals of genotypes GG, GA, and AA are shown in Figure 1. The specificity of PCR products was controlled by amplification of the whole intron 13 using MAOB3FP and MAOB4RP [Kurth et al., 1993] from subjects of representative genotypes GG, GA, and AA, followed by direct sequencing of PCR products across the polymorphic site.

Statistical Analysis

Cases' and controls' allelic distributions were compared by gender. Relative risks were estimated as odds ratios (OR) using logistic regression modeling [Selvin, 1991], controlling for age as a potential confounder. Analyses were performed with the Statistical Analysis System package Version 6.11 for Unix mainframe computers [SAS Institute, Inc., 1993].

RESULTS

Contrary to the previous literature, we found a relative excess of the G allele among cases compared to controls. The overall A:G allelic frequencies were 0.45:0.55 for PD cases and 0.63:0.37 for controls. These data result in a relative risk estimate (odds ratio) of 2.09 (95% confidence interval (CI) 1.18–3.69). Among men, the A:G allelic frequencies were 0.49:0.51 among cases, compared to 0.64:0.36 among controls, giving a relative risk of 1.87 (95% CI, 0.78–4.47) associated with the G allele (Table I). The allelic frequencies (A:G) among women were 0.42:0.58 in cases and 0.62:0.38 among controls. The corresponding relative risks (95% CI) related to the GA and GG genotypes, compared to AA, in women were, respectively, 5.00 (95% CI, 1.13–22.1) and 5.60 (95% CI, 1.01–30.9).

DISCUSSION

Our observation of an apparently elevated PD risk associated with the G allele polymorphism of intron 13 of the MAO-B gene disagrees with the prior literature, which indicated an association with the A allele [Kurth et al., 1993; Ho et al., 1995]. The discrepant findings cannot be attributed to ethnic differences, because all study subjects in ours and in the two studies demonstrating an opposite effect [Kurth et al., 1993; Ho et al., 1995] were Caucasians. The relation with the G allele detected in our study was stronger among women than men. Additionally, we did not observe PD risk differences between heterozygous (GA) and homozygous

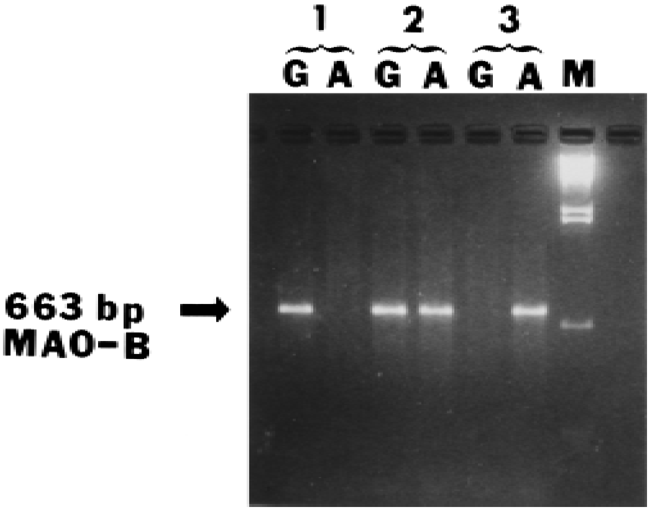

Fig. 1. Ethidium bromide-stained gel showing MAO-B 663-bp-long PCR products obtained from genomic DNA form 3 different subjects **(lanes 1–3).** For each individual, two allele-specific PCR reactions were performed, using the allele-specific reverse primer for amplification of allele G or A, respectively. Subject 1 is of genotype GG, subject 2 is heterozygous GA, and subject 3 is homozygous AA. **Lane M,** molecular weight size marker.

TABLE I. Genotype Distributions of Monoamine Oxidase B in Parkinson Disease Cases and Controls

	Cases (N = 37)		Controls (N = 50)			
Men, genotype	No.	(%)	No.	(%)	OR[a]	(95% CI)
A	18	(48.6)	32	(64.0)	1	
G	19	(51.4)	18	(36.0)	1.87	(0.78–4.47)
	Cases (N = 25)		Controls (N = 29)			
Women, genotype	No.	(%)	No.	(%)	OR[a]	(95% CI)
AA	3	(12.0)	12	(41.4)	1	
GA	15	(60.0)	12	(41.4)	5.00	(1.13–22.1)
GG	7	(28.0)	5	(17.2)	5.60	(1.01–30.9)

[a]OR odds ratio, adjusted for age.

(GG) women, which suggests a dominant mode of expression. The effects of structural variations in the MAO-B gene, including the intron 13 polymorphism, on brain MAO-B enzyme activity are not known [Ho et al., 1994]. Although the variant base we analyzed is located in an intron, and therefore cannot determine amino-acid substitution in the MAO-B enzyme, sequences located in introns, especially in proximity to splice donor and acceptor sites or branch points for mRNA splicing, could be important for mRNA processing. The G/A variant base is located in proximity to the 3′ splicing acceptor site of intron 13 [Ho et al., 1995]; thus, a direct role of this base in modifying MAO-B function cannot be excluded. There is also the possibility that this MAO-B polymorphism is not a marker of PD susceptibility, but rather is in linkage disequilibrium with another gene that directly influences PD risk.

The statistical imprecision of our findings, due to a relatively small number of subjects, limits interpretation somewhat. Much larger population-based studies in diverse ethnic groups will be needed to specify the predictive value for PD risk, if any, of this polymorphism. Correlative studies of brain enzyme function in relation to this, and perhaps to other MAO-B polymorphisms, may prove valuable in identifying PD risk susceptibility profiles. A logical next step would be investigation of MAO-B polymorphisms as modifying factors of associations of PD with cigarette smoking and environmental factors.

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